

WHAT IS CLAIMED:

1. A method for producing a recombinant protein complex having human Factor VIII:C activity, said complex  
5 comprising a first polypeptide homologous to the A domain of human Factor VIII:C, and a second polypeptide having homology to the C domain of human Factor VIII:C, but lacking all/or a substantial portion of the B domain of human Factor VIII:C, which method comprises:  
10 co-expressing in a eukaryotic transformant host cell cultured in a cell growth medium  
(a) a first polynucleotide encoding (i) a first signal sequence capable of directing secretion, and (ii) a first polypeptide comprising  
15 a first region having an amino acid sequence homologous to the A domain of human Factor VIII:C, and  
(b) a second polynucleotide encoding a second polypeptide having an amino acid sequence homologous to the C  
20 domain of human Factor VIII:C; and  
obtaining the secreted recombinant protein complex from said cell medium.
2. A method according to claim 1, wherein not  
25 more than about 5 number% of the amino acids of said amino acid sequences differ from the naturally occurring amino acid sequence of the Factor VIII:C A and C domains.
3. A method according to claim 1, wherein the  
30 amino acid sequence of the polypeptide encoded by the second polynucleotide is the same as the amino acid sequence of amino acids 1649-2322 of human Factor VIII:C.

4. The method of claim 3, wherein the amino acid sequence of the polypeptide encoded by the first polynucleotide is the same as the amino acid sequence of amino acids 1-740 of human Factor VIII:C.

5. The method of claim 3, wherein the amino acid sequence of the polypeptide encoded by the first polynucleotide is the same as the amino acid sequence of amino acids 1-1102 of human Factor VIII:C.

6. The method of claim 3, wherein the amino acid sequence of the polypeptide encoded by the first polynucleotide is the same as the amino acid sequence of amino acids 1-1315 of human Factor VIII:C.

7. The method of claim 3, wherein the amino acid sequence of the polypeptide encoded by the first polynucleotide is the same as the amino acid sequence of amino acids 1-1405 of human Factor VIII:C.

8. The method of claim 1, wherein said first polypeptide further comprises

a second region comprising (a), the N-terminal sequence of the B domain of human Factor VIII:C; (b), a polypeptide spacer of about 3 to about 100 amino acids which has fewer than 5 sites of N-linked glycosylation; and (c), the C-terminal sequence of the B domain of human Factor VIII:C.

9. The method of claim 8 wherein the amino acid sequence of the N-terminal sequence of the B domain of human Factor VIII:C comprises Ser-Phe-Ser-Gln-Asn-Ser-Arg-His-

Pro-Ser-Thr-Arg-Gln-Lys-Gln-Phe-Asn-Ala-Thr.

10. The method of claim 8 wherein said polypeptide spacer comprises a peptide homologous to a human Ig heavy chain hinge region.

11. The method of claim 10 wherein the amino acid sequence of the polypeptide spacer comprises Pro-Pro-Thr-Pro-Pro-Thr.

12. The method of claim 8 wherein the C-terminal sequence of the B domain comprises Pro-Pro-Val-Leu-Lys-Arg-His-Gln-Arg.

13. The method of claim 1 wherein the first polynucleotide further comprises:

(a) a 5' untranslated DNA sequence that increases the expression of the first polypeptide, wherein said 5' untranslated sequence is positioned 5' to said first region, wherein said 5' untranslated DNA is selected from the group consisting of human Factor VIII:C 5' untranslated DNA, SV40 t antigen 5' untranslated DNA, and human cytomegalovirus 1E1 protein 5' untranslated DNA; or

(b) a 3' untranslated DNA sequence that enhances the expression of the first polypeptide, wherein said 3' untranslated sequence is positioned 3' to said polypeptide coding region, wherein said 3' untranslated DNA is selected from the group consisting of human Factor VIII:C 3' untranslated DNA, human tissue plasminogen activator 3' untranslated DNA, and SV40 t-antigen 3' untranslated DNA.

14. The method of claim 1, wherein said second

signal sequence comprises the signal sequence of human  $\alpha$ 1-anti-trypsin.

15. The method of claim 4 wherein said  $\alpha$ 1-anti-  
5 trypsin signal sequence comprises N-Met-Pro-Ser-Ser-Val-Ser-  
Trp-Gly-Ile-Leu-Leu-Leu-Ala-Gly-Leu-Cys-Cys-Leu-Val-Pro-Val-  
Ser-Leu-Ala.

16. The method of claim 1 wherein said second  
10 polynucleotide further comprises:

(a) a 5' untranslated DNA sequence that in-  
creases the expression of said second polypeptide, wherein said  
5' untranslated sequence is positioned 5' to said second  
region, wherein said 5' untranslated DNA is selected from the  
15 group consisting of human Factor VIII:C 5' untranslated DNA,  
SV40 t antigen 5' untranslated DNA, and human cytomegalovirus  
1E1 protein 5' untranslated DNA; or

(b) a 3' untranslated DNA sequence that enhan-  
ces the expression of the second polypeptide, wherein said 3'  
20 untranslated sequence is positioned 3' to said polypeptide  
coding region, wherein said 3' untranslated DNA is selected  
from the group consisting of human Factor VIII:C 3' untrans-  
lated DNA, human tissue plasminogen activator 3' untranslated  
DNA, and SV40 t-antigen 3' untranslated DNA.

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17. The method of claim 1 wherein the eukary-  
otic transformant host cell is a mammalian cell.

18. The method of claim 1 wherein the first  
30 polynucleotide and second polynucleotide are in separate  
expression plasmids.

19. The method of claim 8 wherein the first polynucleotide and second polynucleotide are in separate expression plasmids.

5 20. A protein complex having human Factor VIII:C activity but lacking all or part of the B domain of human Factor VIII:C prepared by the method of claim 1.

10 21. A protein complex having human Factor VIII:C activity but lacking all or part of the B domain of human Factor VIII:C prepared by the method of claim 8.

15 22. A pharmaceutical composition comprising the protein complex of claim 20 and a physiologically acceptable carrier.

20 23. A pharmaceutical composition comprising the protein complex of claim 21 and a physiologically acceptable carrier.

25 24. A method for treating an individual requiring Factor VIII:C activity comprising administering to the individual a sufficient amount of the protein complex of claim 22 to enhance blood clotting activity in the individual.

30 25. A method for treating an individual requiring Factor VIII:C activity comprising administering to the individual a sufficient amount of the protein complex of claim 23 to enhance blood clotting activity in the individual.

26. A DNA composition for transforming a eukaryotic host cell to obtain expression of a recombinant

protein complex having human Factor VIII:C activity, wherein said DNA composition comprises:

5 a first expression cassette, said first expression cassette comprising a first polynucleotide encoding a first signal sequence capable of directing secretion and a first polypeptide comprising a first region having an amino acid sequence homologous to the A domain of human Factor VIII:C; and

10 a second expression cassette, said second expression cassette comprising a second polynucleotide encoding a second signal sequence capable of directing secretion and a second polypeptide having an amino acid sequence homologous to the C domain of human Factor VIII:C.

15 27. The DNA composition of claim 26, wherein said first polynucleotide further comprises:

20 a second region comprising the N-terminal sequence of the B domain of human Factor VIII:C, a polypeptide spacer of about 3 to 40 amino acids which has fewer than 5 sites of N-linked glycosylation, and the C-terminal signal sequence of the B domain of human Factor VIII:C.

25 28. A DNA composition according to claim 26, wherein said first cassette polynucleotide encodes a polypeptide comprising at least about 90% of the amino acid sequence of human Factor VIII:C amino acids 1-740 and said second cassette polynucleotide encodes a polypeptide comprising at least about 90% of the amino acid sequence of human Factor VIII:C amino acids 1649-2332.

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29. A host mammalian cell containing the DNA composition of claim 26.

30. A host mammalian cell containing the DNA composition of claim 27.

5 31. A polypeptide having human Factor VIII:C activity when combined with a polypeptide homologous to human Factor VIII:C Mr 80 K protein, wherein said polypeptide comprises from N-terminal to C-terminal:

10 a first region having an amino acid sequence homologous to the A domain of human Factor VIII:C, and

a second region comprising the N-terminal sequence of the B domain of human Factor VIII:C, a polypeptide spacer of about 3 to 40 peptides which has fewer than 5 sites of N-linked glycosylation, and the C-terminal sequence of the B domain of  
15 human Factor VIII:C.

32. The polypeptide of claim 31, wherein not more than about 5 number% of the amino acids of the amino acid sequence of said first region differ from the naturally occurring amino acid sequence of the Factor VIII:C A domain.  
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33. The polypeptide of claim 31 wherein the amino acid sequence of the polypeptide encoded by the first polynucleotide is the same as the amino acid sequence of amino  
25 acids 1-740 of human Factor VIII:C.

34. The polypeptide of claim 31 wherein the amino acid sequence of the N-terminal sequence of the B domain of human Factor VIII:C comprises Ser-Phe-Ser-Gln-Asn-Ser-Arg-His-Pro-Ser-Thr-Arg-Gln-Lys-Gln-Phe-Asn-Ala-Thr.  
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35. The polypeptide of claim 31 wherein the

amino acid sequence of the polypeptide spacer comprises Pro-Pro-Thr-Pro-Pro-Thr.

36. The polypeptide of claim 31 wherein the C-  
5 terminal sequence of the B domain comprises Pro-Pro-Val-Leu-  
Lys-Arg-His-Gln-Arg.